

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

8

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C12P 7/42	A1	(11) International Publication Number: WO 00/18942 (43) International Publication Date: 6 April 2000 (06.04.00)
(21) International Application Number: PCT/US99/16384 (22) International Filing Date: 20 July 1999 (20.07.99) (30) Priority Data: 09/161,129 25 September 1998 (25.09.98) US (71) Applicant: GENERAL ELECTRIC COMPANY [US/US]; 1 River Road, Schenectady, NY 12345 (US). (72) Inventors: JOHNSON, Bruce, Fletcher; 36 Irving Road, Scotia, NY 12302 (US). AMARATUNGA, Mohan; 6 Hampton, London Square, Clifton Park, NY 12065 (US). LOBOS, John, Henry; 33 Glen Street, Ballston Spa, NY 12020 (US). (74) Agents: SNYDER, Bernard et al.; General Electric Company, 3135 Easton Turnpike W3C, Fairfield, CT 06431 (US).		(81) Designated States: JP, SG, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: METHOD FOR INCREASING TOTAL PRODUCTION OF 4-HYDROXYBENZOIC ACID BY BIOFERMENTATION USING AN ANION EXCHANGE RESIN (57) Abstract <p>This invention pertains to a method of production of 4-hydroxybenzoic acid, with purification and recovery of 4-hydroxybenzoic acid directly from the fermentation medium of a cultured organism, in the presence of other chemical species. Ion exchange resin is used during the fermentation process to recover the chemical product without the necessity of separating the biomass from the fermentation supernatant by filtration after fermentation is complete and then treating the filtrate to recover the product. This reduces or eliminates the need for filtration steps and eliminates the need for energy-intensive water removal processes. Furthermore, the removal of the product during the fermentation dramatically increases the production of the product during the fermentation process. This method therefore can both increase the amount of product produced in a single fermentation run and reduce the per unit cost of its biocatalytic production.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

- 1 -

METHOD FOR INCREASING TOTAL PRODUCTION OF 4-HYDROXYBENZOIC ACID BY BIOFERMENTATION USING AN ANION EXCHANGE RESIN

5 This invention was made with Government support under Government Contract No. 70NANB5H1135, awarded by the National Institute of Science and Technology. The Government has certain rights in this invention.

10 **TECHNICAL FIELD**

 This invention relates to a method for producing 4-hydroxybenzoic acid (parahydroxybenzoic acid, PHB) using biofermentation. This method increases the potential total production of PHB and recovery of PHB from the fermentation medium. In
15 particular, the invention is directed toward a method for removing PHB from a fermentation mixture by continuous exposure and binding to an anion exchange resin during its biocatalytic production.

BACKGROUND ART

20 Organic synthesis of chemical intermediates and raw materials important to the polymer and chemical industries can be a major portion of the cost of production of the finished product made from them. Often, starting materials are synthesized from petroleum products which are non-renewable, expensive, and may be hazardous.
25 In addition, the cost of petroleum products is dependent on world markets and other factors which can not be controlled. Another factor which increases costs in the production of organic molecules by a chemical synthetic route is the resulting waste stream of unwanted byproducts and contaminated solvents, the disposal of which is
30 expensive and hazardous. Yet, for most chemical starting materials and intermediates, there is no other practical alternative to chemical synthesis.

 Production of starting materials by biofermentation is an alternative which uses renewable resources and creates much less
35 hazardous waste. The fermentative production of a few chemicals has

- 2 -

been reported (W. Crueger and A. Crueger, Biotechnology: A Textbook of Industrial Microbiology, Sinauer Associates, Sunderland, MA, pp. 124-174 (1990); B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, second edition, Stockton Press, NY, pp. 243-364 (1991)) and commercialized. In a recent review on improving the production of aromatic compounds in *Escherichia coli* (*E. coli*) by metabolic engineering, an extensive array of compounds is detailed (A. Berry, *Tibtech* 14:250-256 (1996)).

Unfortunately, this is not a commercially feasible alternative for a great many desirable molecules because the molecule of interest is produced in such small amounts by the biocatalytic organisms. Even organisms genetically engineered to produce enhanced amounts of the desired molecule often do not produce concentrations of the product great enough to justify the investments necessary to develop a commercial biofermentation process. This can be particularly true when the product is toxic to the cells or is regulated by a negative feedback mechanism, thereby limiting the potential concentration of the product in the fermentation medium.

At the same time, one of the greatest challenges to the development of a biofermentative process is the separation, recovery, and purification of the product from the fermentation medium at relatively low concentrations and in the presence of other chemical species. Removal of the desired metabolites from fermentation mixtures is typically performed after the termination of the fermentation process. Generally, the process involves a first filtration step to separate the biomass and yield a clarified solution from which product is removed. Often, the process then includes an energy-intensive water-removal step to concentrate the desired product or products. The product of interest must then be separated from the other molecules in the fermentation medium, for example metabolic byproducts and culture medium constituents.

This separation and purification may be performed by any of a number of techniques known in the art of biochemistry, such as electrodialysis, electrophoresis, sedimentation, solvent extraction, precipitation, or distillation. For example, lactic acid may be purified

- 3 -

from fermentation medium using electrodialysis (P. Vonktaveesuk *et al.*, J. Ferment. Technol. 77:508-512 (1994); Y. Nomura *et al.*, J. Ferment. Technol. 71:450-452 (1991) or extraction (V. Yabannavar and D. Wang, Biotechnol. Bioeng. 37:1095-1100 (1991)). Acetic acid
5 has also been purified after biofermentation using electrodialysis (S. Shang and K. Toda, J. Ferment. Technol. 77:288-292 (1994)).

Standard techniques are not commercially viable unless the product is present in the fermentation broth in relatively high concentrations, therefore they are limited in usefulness to products
10 which are produced in relatively high concentrations in the fermentation medium. Even when feasible to do in the laboratory, the product recovery steps add to manufacturing costs often preventing the commercialization of the fermentative production of a wide range of chemicals. For production of these compounds to be cost-effective,
15 more selective and less expensive techniques must be developed to remove and purify low concentrations of product.

For example, 4-hydroxybenzoic acid (PHB), a key monomer in the synthesis of liquid crystalline polymers (LCPs) with additional utility as a chemical intermediate for the manufacture of paraben
20 preservatives and other products, is currently produced by organic chemical synthesis and costs approximately \$2.40/lb. The high cost of PHB contributes significantly to the high cost of LCPs and thus limits the applications to which LCPs can be put commercially. The biofermentative production of PHB from glucose can potentially reduce
25 the manufacturing costs of LCPs now, and in the future by insulating PHB from potential increasing petrochemical costs as oil reserves are depleted. A key challenge in the fermentative production of this chemical, however, is the isolation of the desired product from the aqueous fermentation mixture at relatively low concentrations (<10%
30 w/v) in the presence of other chemical species (W. Crueger and A. Crueger, Biotechnology: A Textbook of Industrial Microbiology, Sinauer Associates, Sunderland, MA, pp. 111-123 (1990); B. Atkinson and F. Mavituna, Biochemical Engineering and Biotechnology Handbook, second edition, Stockton Press, NY, pp. 905-1022 (1991)).

- 4 -

PHB can be made from glucose using *E. coli*, which produces the chemical as a minor metabolite and excretes the product into the medium at levels of less than 2 mg/L. The biosynthetic pathway in *E. coli* is shown in Figure 1. A significant number of steps are required in the metabolic pathway from 3-deoxy-*D*-arabino-heptulosonate-7-phosphate (DAHP) to chorismic acid, variously termed the chorismic acid, aromatic amino acid, or shikimic acid pathway.

Genetically engineered biocatalysts are known to produce chemicals like PHB. However, the amounts produced by these strains are not optimal for recovery by the prior art methods. There is a need, therefore, for a method to increase the recovery of PHB commercially produced using biofermentative methods.

SUMMARY OF THE INVENTION

Accordingly, this invention provides a method for producing 4-hydroxybenzoic acid by biofermentation comprising providing a culture of biocatalyst which produces 4-hydroxybenzoic acid in a fermentation medium; allowing the biocatalyst to produce 4-hydroxybenzoic acid by fermentation; removing 4-hydroxybenzoic acid from the fermentation medium during at least a portion of the fermentation by passing the fermentation medium through an anion exchange resin which binds 4-hydroxybenzoic acid for at least a portion of the biofermentation; and extracting the anion exchange resin to remove bound 4-hydroxybenzoic acid. This results in superior recovery of the product and higher production of the product by the biocatalyst.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic diagram showing the biosynthetic pathway for the production of chorismate, PHB and aromatic amino acids from glucose in *E. coli*.

Figure 2 depicts the binding of PHB, phenylalanine, and tyrosine to anion exchange resin in minimal culture medium in the presence of the competing amino acids and in the presence or absence of 7.4 mM acetate.

- 5 -

Figure 3 shows the PHB, tyrosine, and phenylalanine produced during a biofermentation in the presence of anion exchange beads.

DESCRIPTION OF THE INVENTION

5 According to the method of the invention, the biocatalysis product, PHB is continuously removed during at least part of the fermentation process. Biocatalysis may be performed by any fermentation method available in the prior art, using any cell capable of producing the molecule. Wild type or genetically engineered cells may
10 be used with this method, for example an *E. coli* strain such as JP01B/pMCP2, a genetically engineered *E. coli* strain containing the multicopy plasmid pMCP2 which overproduces chorismic acid. Fermentation is desirably carried out in a fermentor, or any suitable container such as shake flask, using any medium which provides the
15 correct nutritional support and sufficient raw materials for production of the desired final product.

 Anion exchange resin may be prepared by conversion to the phosphate form by prewashing with phosphate buffer and sterilization, for example by autoclaving or rinsing with alcohol. The resin is
20 preferably placed in a separatory flask or funnel to minimize loss of the resin from the column to the stirred fermentor, but may be placed in any suitable column or other container. During at least part of a fermentation process, the fermentation medium is passed through the bed of anion exchange beads, preferably in an upwards direction, and
25 back into the fermentor. The passage of culture medium through the beads may be performed during any portion of the fermentation process, preferably from 6 hours after addition of yeast to the reactor until the end of the fermentation process. The culture medium also may be passed through the beads throughout the entire fermentation
30 process. The fermentation mixture is preferably pumped through the resin bed using a peristaltic pump to minimize cell disruption, but any suitable pump may be used. As the anion exchange beads become saturated with PHB during the fermentation, replacement with fresh beads or the initial use of a greater quantity of beads will further
35 improve the production and removal of PHB from the medium.

- 6 -

The anion exchange resin for use with this invention may be a weakly basic or strongly basic type resin. If a weakly basic resin is used, the fermentation medium is desirably adequately acidic to protonate the basic group on the resin, for example about pH 7 to protonate a tertiary amine. Preferably, the anion exchange resin is a strongly basic cross-linked polystyrene-based resin such as IRA-400 (Amberlite®). The beads used with this invention desirably should have about 0.3 mmols to about 1.3 mmols cationic binding sites per mL fermentation medium, and preferably about 0.6 mmols to about 0.9 mmols cationic binding sites per mL fermentation medium.

During fermentation, PHB becomes bound to the ion exchange resin as it is passed through the anion exchange beads, and fermentation medium depleted of PHB is returned to the fermentor. The concentration of PHB in the fermentor, thereby rapidly decreases once circulation through the anion exchange resin begins, and increases more slowly over the course of the fermentation process than in fermentations performed without exposure to anion exchange beads.

Once fermentation is completed, or once saturated anion exchange beads are removed from the system and replaced, the PHB is preferably recovered from the resin by extracting with acidic ethanol or sodium chloride in a water/ethanol mixture. Any pH, salt or chaotrope treatment which conveniently releases the PHB from the beads may be used. Anion exchange resin may be reused after the PHB is unbound, by first washing with phosphate buffer and resterilizing.

The following provide examples of fermentative processes to illustrate the invention, and are not intended to be limiting.

EXAMPLES

1. Determination of Competitive Binding of PHB to Anion Exchange Resin

Anion exchange resin, IRA-400(Cl) was converted to the phosphate form by washing the resin with 10 times the resin bed volume of 100 mM phosphate buffer, pH 7. Binding of PHB to the

- 7 -

resin was performed in vials containing 1 ml basic culture medium (1 mM MgSO₄, 22 mM KH₂PO₄, 69 mM K₂HPO₄, 33 mM NaCl, and 39 mM (NH₄)₂SO₄), with 3.2 mM PHB, 3.7 mM tyrosine, and 3.5 mM phenylalanine. One set of vials also contained 1 ml bed volume of
5 anion exchange resin, phosphate form (equivalent to 1.4 mmoles cationic sites), and one set also contained the anion exchange resin as above plus 7.4 mM sodium acetate. The total volume of all vials was brought to 10 ml with basic culture medium. The vials were shaken for 3 hours, then rested to allow the beads to settle. The supernatant was
10 analyzed by HPLC to determine the amount of PHB, tyrosine, and phenylalanine remaining in solution.

The results are shown in Figure 2. In the absence of anion exchange resin, nearly 100% of the PHB recovered was in solution. In the presence of resin, however, over 97% of the PHB originally present
15 in solution was bound. Only 30% and 15% of tyrosine and phenylalanine respectively was bound to the resin, demonstrating a high degree of specificity under the conditions tested. Even in the presence of excess competing anionic species (acetate ion), the anion exchange resin effectively adsorbed PHB, binding over 97% of the
20 PHB in solution. This demonstrates the efficacy of this resin for the adsorption of PHB from aqueous solutions, even at low PHB concentration (3.2 mM).

Using this method, other ion exchange resins may be tested for the ability to specifically bind PHB under the fermentation conditions
25 under which it will be produced.

2. Enhanced Fermentative Production of PHB.

Fermentative production of PHB was demonstrated in fermentors using the biocatalyst JP01B/pMCP2. JP01B is a strain of
30 *E. coli* genetically engineered to overproduce chorismic acid. pMCP2 is a multicopy plasmid designed to overproduce *ubiC*, the gene coding for *E. coli* chorismate pyruvate lyase, the enzyme that converts chorismic acid to PHB.

Inocula for the fermentations were revived from frozen culture stock stored at -80°C in 30% glycerol. The cultures were grown in 5 ml
35

- 8 -

tubes of Luria broth containing 100 mg/L carbenicillin for 8 h before transferring a 1% volume into a 1 liter baffled shake flask containing 300 ml SMM medium (10g/L glucose; 12.0 g/L K_2HPO_4 ; 3.0 g/L KH_2PO_4 ; 1.0 g/L $MgCl_2 \cdot 6H_2O$; 4 g/L $(NH_4)_2SO_4$; 0.5 g/L $CaCl_2 \cdot 2H_2O$; 0.4 mg/L $CuSO_4 \cdot 5H_2O$; 0.5 mg/L $ZnSO_4 \cdot 7H_2O$; 25.0 mg/L $MnSO_4 \cdot H_2O$; 1.0 mg/L $CoCl_2 \cdot 6H_2O$; 0.2 mg/L sodium molybdate; 50 mg/L $FeSO_4 \cdot 7H_2O$; 50 mg/L sodium citrate; 100 mg/L carbenicillin; and 1.0 g/L Amberex® 695 yeast extract). The baffled shake flasks were incubated 37°C with vigorous shaking (300 rpm) for 15 hours before addition to the fermentors.

Each fermentor contained an initial volume of 3.5 liters of SMM medium before inoculating with 300 ml of an overnight culture of *E. coli* JP01B containing the pMCP2 plasmid. Carbenicillin (100 mg/L) was added to the medium initially to maintain cells containing the ampicillin resistance gene which is present on the pMCP2 plasmid. The initial glucose concentration was 0.5% (w/v). During fermentation, glucose was added using the DO-Stat method (K. Konstantinov *et al.*; Biotechnol. Bioeng. 36:750-758 (1990)) to control the rate of feeding. The dissolved oxygen (DO) was set at 20% of saturation (about 2 ppm O_2) by sparging with air. As the DO increased above the set point, the glucose feed increased to bring it back to 20%, and when the DO dropped below the set point, glucose feeding was reduced. The cultures were kept at 35°C, and the pH was controlled at 7.0 with NH_4OH and H_2SO_4 . The cultures were continuously agitated at 1000 rpm. Fermentations were run simultaneously under nearly identical conditions for over 50 hours.

One fermentation employed a culture recycle through Amberlite® IRA- 400(Cl) anion exchange resin for the continuous removal of PHB during biocatalyst growth and PHB production. The other did not. For the fermentation employing beads, beginning after the initial 5 hours of growth, the culture was continuously circulated through a 2 L conical funnel containing a 500 ml bed volume of anion exchange resin. The anion exchange resin had been converted to the phosphate form by washing with 10 times the resin bed volume of 100 mM phosphate buffer, pH 7 and sterilized with an ethanol rinse,

- 9 -

followed by a sterile water rinse to remove the ethanol. The culture was pumped from the fermentor up through the bottom of the funnel at a rate of 400 ml/min to create a fluidized bed of the beads. The beads settled out of the top 1-2 inches of the culture medium in the bead reactor (funnel), creating an essentially bead-free culture medium supernatant. This culture medium was continuously returned to the fermentor. At the pumping rate employed, the entire culture volume was cycled through the beads every 10 minutes. After the fermentation was complete, the cell culture medium was rinsed off the beads with water before the beads were extracted to recover the PHB. The PHB was quantitatively recovered from the IRA-400 resin by soxhlet extraction using 3L 95% ethanol containing one molar equivalent of trifluoroacetic acid. PHB concentration in the fermentation supernatants was determined by HPLC.

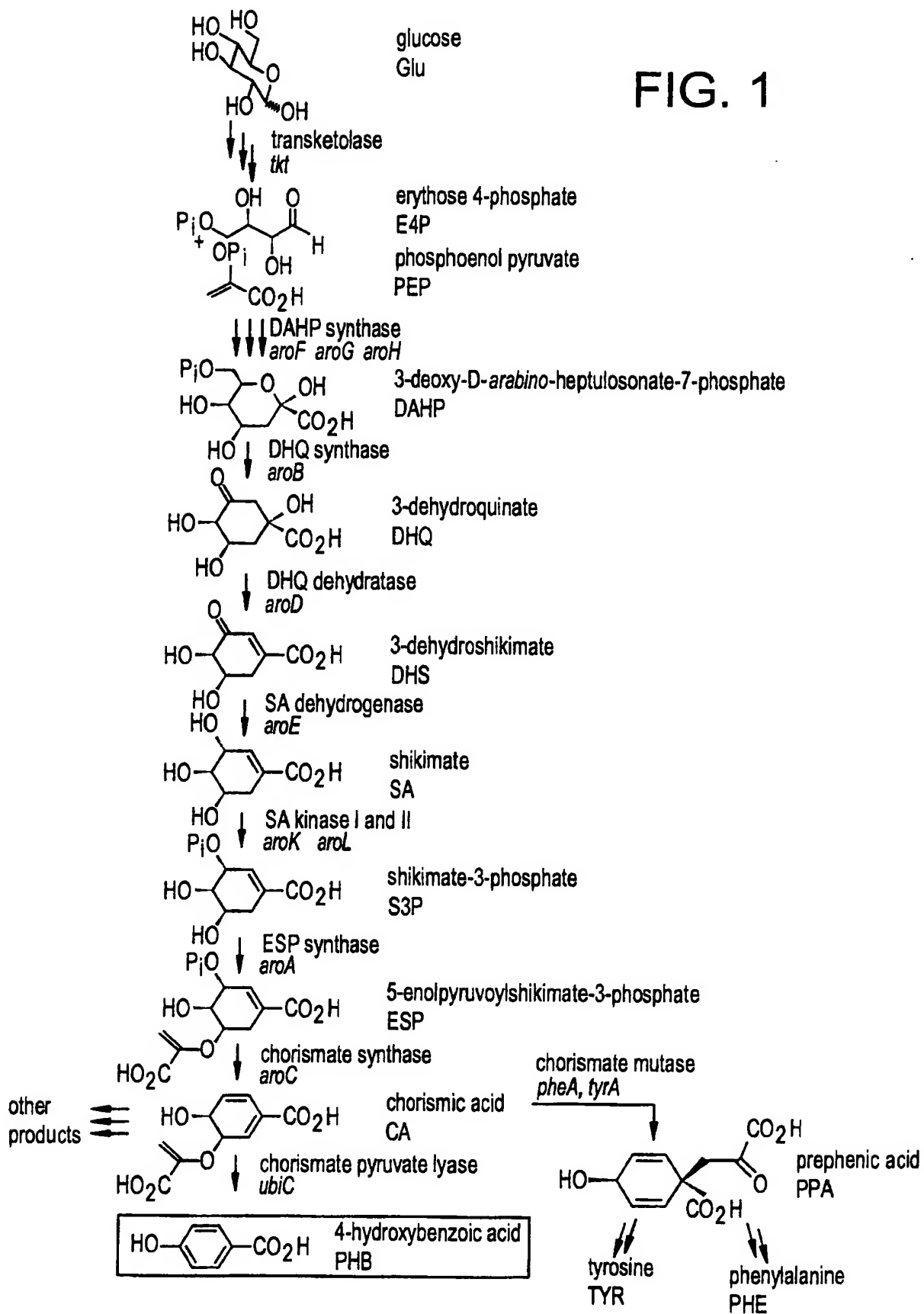
Results of the fermentation using beads according to the invention are reproduced in Figure 3. The PHB recovered from the resin at the end of the fermentation was normalized to the final fermentation volume and is reported as a single data point (14.2 g/L). The concentration of PHB in the medium of the fermentation employing anion exchange beads after 55 hours of fermentation was 8.7 g/L, for a total equivalent recovery of 22.9 g/L (see Figure 3). The best fermentation run under similar conditions without anion exchange resin produced 6 g/L PHB in the fermentation medium (data not shown).

- 10 -

CLAIMS

1. A method for producing 4-hydroxybenzoic acid by biofermentation, comprising:
 - a) providing a culture of biocatalyst which produces 4-hydroxybenzoic acid in a fermentation medium;
 - 5 b) allowing the biocatalyst to produce 4-hydroxybenzoic acid by fermentation;
 - c) removing 4-hydroxybenzoic acid from the fermentation medium by continuously passing the fermentation medium through an anion exchange resin which binds 4-hydroxybenzoic acid for at least a portion of the biofermentation; and
 - 10 d) extracting the anion exchange resin to remove bound 4-hydroxybenzoic acid.
2. A method according to claim 1, wherein the biocatalyst is *E. coli*.
3. A method according to claim 2, wherein the *E. coli* is wild- type *E. coli*.
4. A method according to claim 2, wherein the *E. coli* is genetically engineered *E. coli*.
5. A method according to claim 4, wherein the *E. coli* is JP01B/pMCP2.
6. A method according to claim 1, wherein the fermentation medium is passed through the anion exchange resin using a peristaltic pump.
7. A method according to claim 1, wherein the direction of flow of the fermentative medium through the anion exchange medium is in an upward direction.
8. A method according to claim 1, wherein the anion exchange resin is contained in a conical-shaped container.

FIG. 1



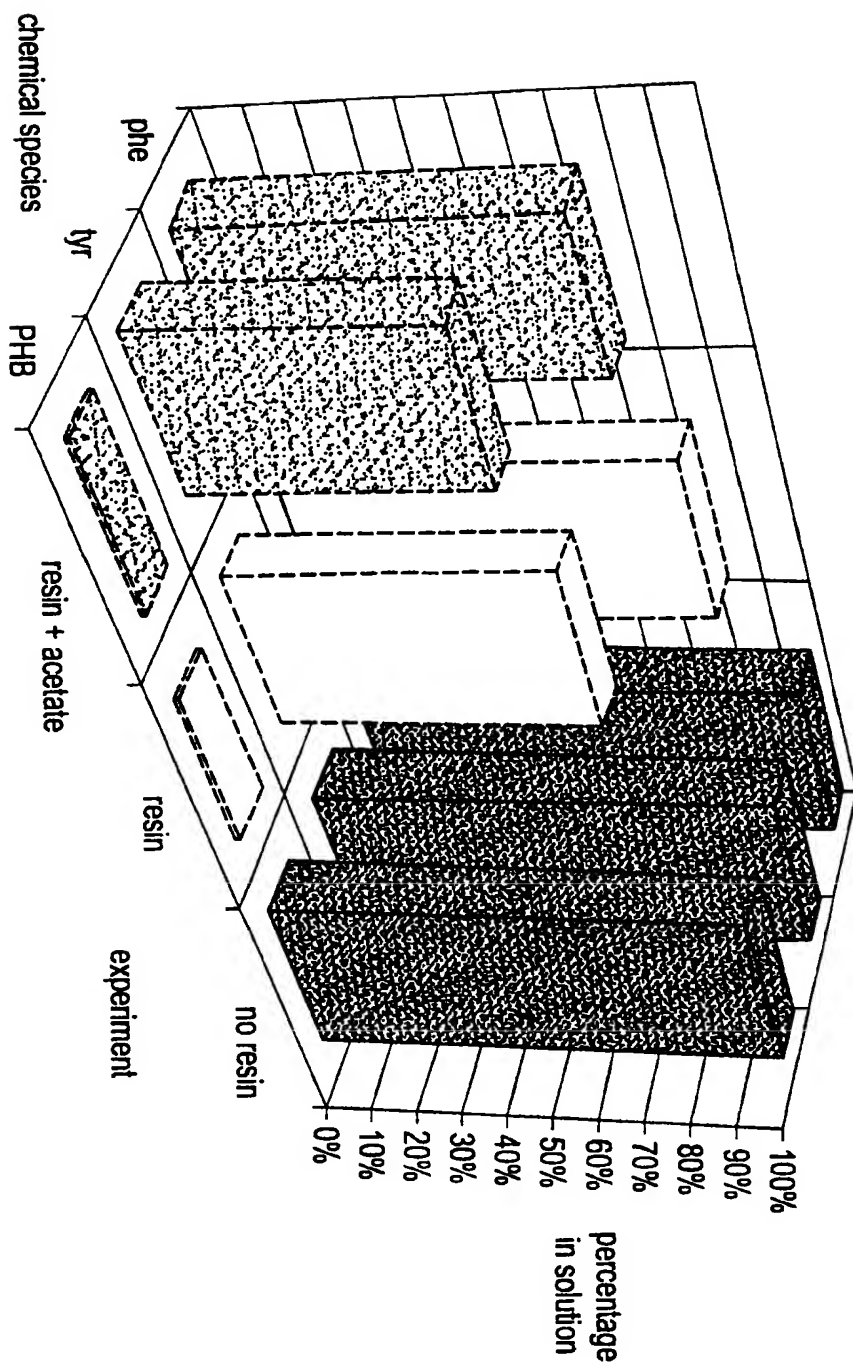
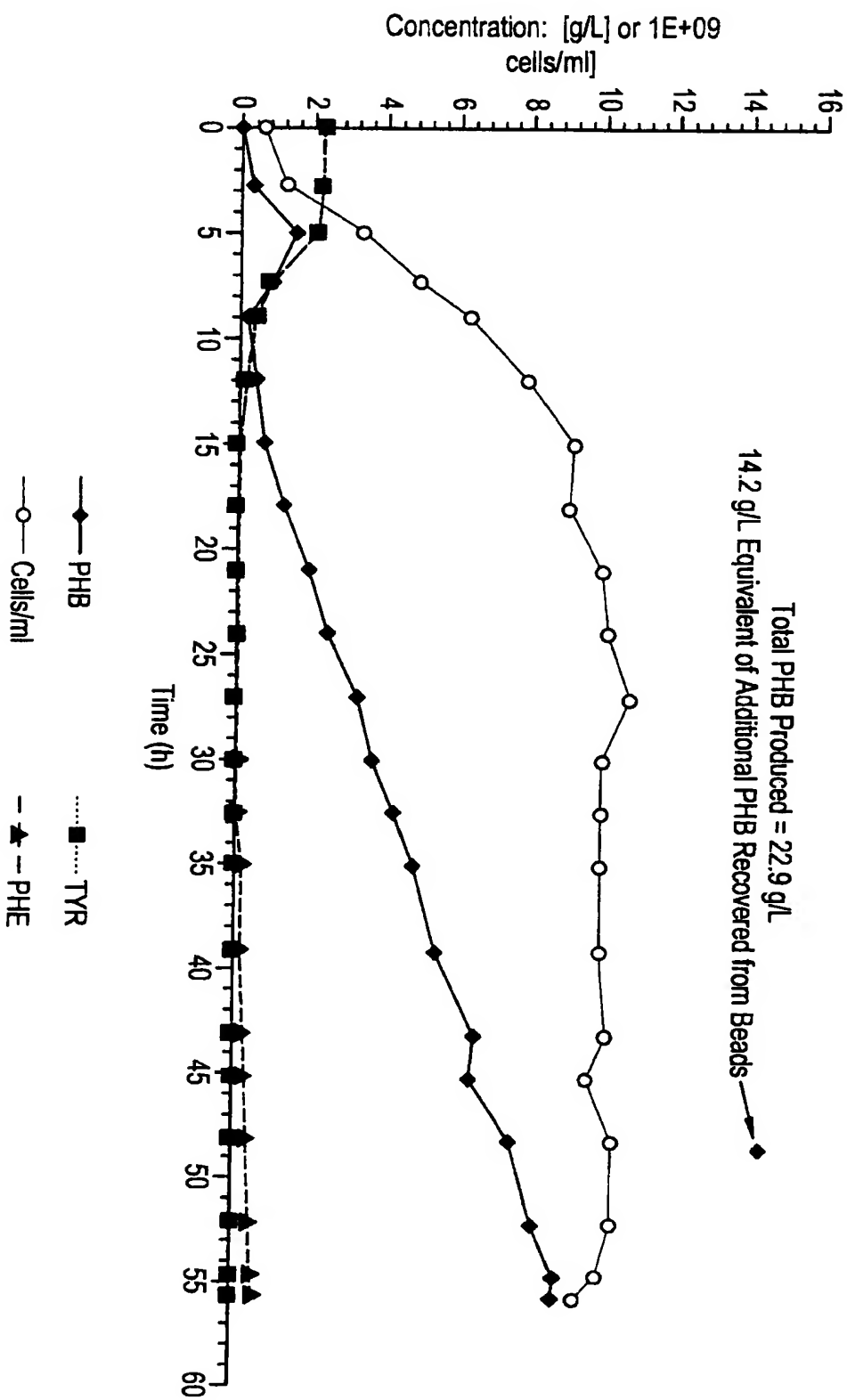


FIG. 2

3 / 3



INTERNATIONAL SEARCH REPORT

In: ational Application No

PCT/US 99/16384

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12P7/42

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 111 310 A (SUN OIL COMPANY) 24 April 1968 (1968-04-24) page 2 -page 5; claim 1; example 4	1-8
Y	CHEMICAL ABSTRACTS, vol. 70, no. 25, 23 June 1969 (1969-06-23) Columbus, Ohio, US; abstract no. 114836, MIHARA, KEISUKE ET AL: "Separation of p-(2-hydroxyethoxy)benzoic and p- hydroxybenzoic acids" XP002122977 abstract & JP 44 002456 B (POLYESTER ETHER * DEVELOPMENT CO., LTD.) 1 February 1969 (1969-02-01) -/-	1-8

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

17 November 1999

Date of mailing of the international search report

03/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5618 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3018

Authorized officer

Douschan, K

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 99/16384

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CHEMICAL ABSTRACTS, vol. 90, no. 25, 18 June 1979 (1979-06-18) Columbus, Ohio, US; abstract no. 203639, TANAKA, MASAYA ET AL: "Anion-exchange separation of the isomers of hydroxybenzoic acid" XP002122978 abstract & TOTTORI DAIGAKU KOGAKUBU KENKYU HOKOKU (1978), 9(1), 88-95 , _____	1-8
Y	US 4 851 573 A (UOP) 25 July 1989 (1989-07-25) the whole document _____	1-8
Y	DE 28 52 596 A (GLAXO GROUP LTD.) 7 June 1979 (1979-06-07) the whole document _____	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 99/16384

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
GB 1111310 A		BE 690898 A DE 1593461 A FR 1503381 A JP 51032714 B NL 6617111 A	08-06-1967 16-07-1970 08-02-1968 14-09-1976 09-06-1967
JP 44002456 B		NONE	
US 4851573 A	25-07-1989	CA 1303061 A US 4720579 A JP 1191691 A JP 1727058 C JP 4012709 B US 4851574 A US 4924027 A US 5068419 A CN 1034392 A,B IN 167770 A	09-06-1992 19-01-1988 01-08-1989 19-01-1993 05-03-1992 25-07-1989 08-05-1990 26-11-1992 02-08-1989 15-12-1990
DE 2852596 A	07-06-1979	BE 872535 A CH 641184 A FR 2411197 A GB 2009734 A,B IE 47610 B IT 1106371 B JP 54101490 A NL 7811868 A US 4205165 A	05-06-1979 15-02-1984 06-07-1979 20-06-1979 02-05-1984 11-11-1985 10-08-1979 08-06-1979 27-05-1980